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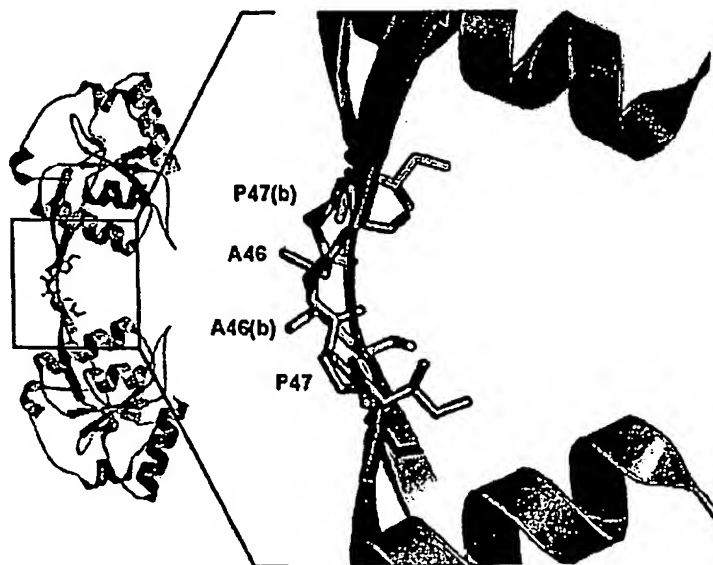
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(54) Title: MODULATION OF ENZYME ACTIVITY BY CHANGING RECIPROCAL STEREO-GEOMETRIC POSITIONS OF TWO CATALYTIC CENTERS IN AN ENZYME



(57) Abstract: Compositions and methods are provided that relate to a modified DNA cleaving enzyme having at least 35% amino acid sequence identity with T7 Endo I. The modified enzyme includes two catalytic centers separated by a  $\beta$ -bridge where the  $\beta$ -bridge contains at least one mutation having an effect of altering enzyme cleavage activity compared to the unmodified enzyme. Activities associated with the modified DNA cleaving enzyme that can be modulated in different reaction conditions include at least one of: (a) non-sequence specific nicking activity; (b) cleaving the second strand of a duplex DNA at a preexisting nick site to produce a linear duplex with a single strand overhang; (c) non-sequence specific DNA cleavage; (d) cleaving DNA flanking a mismatch; and (e) cleavage at a cruciform structure in a DNA duplex.



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